Association between TNFA-308 G/A polymorphism and sensitization to para-phenylenediamine

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Original article

Association between *TNFA-308 G/A* polymorphism and sensitization to *para*-phenylenediamine: a case–control study

Background: *Para*-phenylenediamine (PPD) and related chemicals are common contact sensitizers, frequently causing allergic contact dermatitis (ACD). The cytokine tumor necrosis factor-alpha (TNF-α) plays a key role in contact sensitization.
Methods: In this case–control study, we evaluated the distribution of variations in the regulatory region of the gene for TNF-α (*TNFA-308 G/A*) in 181 Caucasian individuals with a history of ACD and sensitization to PPD and 161 individuals with no history of sensitization to PPD.
Results: The frequency of *GA* or *AA TNFA* genotypes was significantly higher in individuals sensitized to PPD than in age- and gender-matched controls giving an odds ratio (OR) of 2.16 (95% confidence interval, CI: 1.35–3.47; $P = 0.0016$). This relation was even more pronounced when restricting cases to females over 45 years (OR = 3.71; 95% CI: 1.65–8.31; $P = 0.0017$) *vs* younger females (less than or equal to 45 years; OR = 2.41; 95% CI: 1.03–5.65; $P = 0.044$) or males (OR = 1.05; 95% CI: 0.449–2.47; $P = 1.0$). In addition, a logistic regression model revealed a significant effect for *TNFA-308 AA* and *AG vs GG* genotype (point estimate = 2.152; 95% Wald CI: 1.332–3.477).
Conclusions: These findings suggest a possible role for the *TNFA-308* genetic polymorphism as a susceptibility factor for chemically induced ACD.

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Key words: allergic contact dermatitis; *para*-phenylenediamine; polymorphism; sensitization; tumor necrosis factor-alpha.

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Para-phenylenediamine (PPD) is a *small* chemical that is widely used as ingredient in various products including hair dyes. Sensitization to PPD is a well-known cause for allergic contact dermatitis (ACD), especially among hairdresser (1, 2). Moreover, ACD because of PPD containing skin paints (temporary tattoos) is increasingly reported (3). Up to now, it is not completely understood why certain individuals are more likely to develop ACD after exposure to PPD than others. Genetic factors influencing the sensitization or reactivation process may have impact on the individual susceptibility.

Previously, it has been shown that PPD and PPD analogs activate dendritic cells (DCs) (4, 5), which is regarded as a major step during the development of cutaneous immune responses. Activated DCs including epidermal Langerhans cells migrate and deliver potentially foreign signals to draining lymph nodes to stimulate naïve T-cells (6, 7). This activation and migration process depends on several chemokine and cytokine signals, mainly provided by tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) (8, 9). In contrast to irritants, chemical allergens have been found to stimulate DCs *in vitro* to produce high levels of TNF-α (10). Both TNF-α receptors, p55TNFR and p75TNFR, are essential for the development of a complete delayed type hypersensitivity (DTH) reaction. DCs lacking the p55TNFR have a defective allergen uptake and DCs of p75TNFR-deficient mice exhibit diminished migration into regional lymph nodes after contact with allergens (11). Additionally, the blockade of TNF-α-induced migration can provide protection against contact allergy in mice (12). The importance of TNF-α is further stressed by studies demonstrating that only after TNF-α-induced detachment of DCs from neighboring keratinocytes, cytokines such as IL-16 support the emigration of DCs, whereas anti-inflammatory cytokines such as IL-10 inhibit this process most likely by downregulation of TNF-α production (13). Taken together, these results indicate that

TNF-α plays a pivotal role in a network of chemokines and cytokines involved in sensitization and ACD caused by the small chemical PPD.

A single nucleotide polymorphism (SNP; guanine to adenine, *TNFA-308 G/A*), located at nucleotide-308 upstream of the *TNFA* transcription start site, is known for its strong influence on the promoter activity of the *TNFA* gene and has been associated with various inflammatory disorders including allergic and irritant contact dermatitis (14–20). The less common A allele confers an increased transcription capacity of the *TNFA* gene resulting in an enhanced production of TNF-α (21, 22). In this case–control study, we investigated an association between *TNFA-308* polymorphism and sensitization to small chemicals such as PPD.

Materials and methods

Cases and controls

Cases consisted of 181 unrelated Caucasian individuals from Germany and the Netherlands with a history of ACD and sensitization to PPD based on a positive patch-test reaction [1 + to 3 +, according to the International Contact Dermatitis Research Group's (ICDRG) classification] at the second reading (72 h or 96 h). In agreement with other studies, the majority of cases was oligosensitized. Among those with strong reaction to PPD (+ + or + + +), additional positive reactions primarily to other *para*-compounds, such as *para*-tolulenediamine and disperse orange, as well as to nickel, were more frequent. Because of the small numbers of mono- and polysensitized individuals, they were not separately analyzed nor combined into subgroups. Controls consisting of 161 unrelated Caucasians with no history of sensitization to PPD or ACD were age- and gender-matched to the cases. The local ethics committee had approved this study. All subjects gave written informed consent and donated blood.

Genotyping for the *TNFA-308 G/A* genetic polymorphism

Genomic DNA was isolated from the venous blood sample or serum using a commercially available kit (QIAamp Bloodkit; Qiagen, Hilden, Germany). One microliter (20–80 ng) genomic DNA was used for typing. Genotyping for the *TNFA-308 G/A* genetic polymorphism was performed in a real-time polymerase chain reaction (PCR) assay with specific fluorescence-labeled hybridization probes as described earlier (23). The *G* to *A* transition in the promoter region defines the rare allele associated with an elevated expression and release of TNF-α protein. In brief, 0.5 μM of the primers (sense: 5′-AAGGAAACAGACCACAGACCTG; antisense: 5′-GGTCTTCTGGGCCACTGAC), 3 mM $MgCl_2$ as well as 0.2 μM of the detection FITC (Y)-labeled hybridization probe 5′-AACCCCGTCCCCATGCC (specific for the G allele) covering the polymorphic position and annealing next to the anchor probe (LC Red (X)-5′-CAAAACCTATTGCCTCCATTTCTTTTGGGGAC). PCR conditions included 95°C for 2 min and 40 cycles of 95°C for 5 s, 57°C for 5 s, and 72°C for 10 s followed by melting curve analysis.

Statistical analysis

All genotypes were tested for the Hardy–Weinberg equilibrium. The *P*-values obtained by Fisher's two-sided exact test were used to test for associations between contact sensitization and polymorphisms. ORs and 95% CIs were calculated from the ratio of variant *vs* common genotypes in cases and controls, or other strata, respectively. Logistic regression was calculated for differences regarding polymorphism, age, and gender between cases and controls. All tests were analyzed using the SAS program (SAS Institute Inc., Cary, NC, USA).

Results

A total of 181 cases and 161 age- and gender-matched controls underwent successful genotyping for the -308 (*G* to *A*) genetic polymorphism in the *TNFA* gene. Both groups consisted of 70% females and 30% males. The average age of all subjects was 45 ± 16 (mean value ± SD) years. The *TNFA GG* homozygous genotype was present in 110 (61%) cases *vs* 124 (77%) controls, the *TNFA GA* heterozygous genotype was observed in 62 (34%) cases *vs* 34 (21%) controls, whereas the *TNFA AA* homozygous genotype was found in 9 (5%) cases *vs* 3 (2%) controls. The allele frequencies observed in the controls in this study were comparable to published data for mid-Europeans. Consequently, genotype frequencies in the controls and cases fulfilled the Hardy–Weinberg equilibrium. As shown in Table 1, the frequency of the rare A allele was significantly higher in cases than in controls (22.1% *vs* 12.4%; $P = 0.0009$). Frequencies for the predominant allele *G* were 87.5% in the controls and 77.9% in cases.

The distribution of *TNFA* genotypes in cases differed significantly from that in the controls (see Table 2), comparing *TNFA A* carriers (*GA* or *AA* genotypes) with noncarriers (*GG* genotype) (OR = 2.16; 95% CI: 1.348–3.47; $P = 0.0016$) and heterozygous *TNFA GA* carriers with noncarriers (*GG* genotype; OR = 2.06; 95% CI: 1.26–3.36; $P = 0.0037$). Subgroup analysis revealed no significantly increased risk for males only (OR = 1.05; CI: 0.45–2.47). In contrast, the magnitude of association between *TNFA A* carriers (*GA* and *AA* genotypes) and individuals sensitized to PPD was increased when restricting the analysis to females (123 cases and 112 controls). The adjusted OR was 2.93 (95% CI: 1.64–5.24; $P = 0.00027$). Next, we studied the impact of age (less than and greater than median) in females. Again, we found an increased number of *TNFA A* carriers in both groups of female cases compared with age- and gender-matched female controls, with a stronger association in

Table 1. Allele frequency for *TNFA-308* in PPD-sensitized cases and controls

	TNFA-308 allele		
Individuals	A%	G%	*P*†
Cases	22.10	77.90	0.000883
Controls	12.42	87.58	

†Two-sided Fisher's exact test.

Table 2. Association between *TNFA-308 GG, GA,* and *AA* genotypes and PPD-sensitized cases and controls

	Total	GG	GA+AA		
Individuals	*n*	*n* (%)	*n* (%)	OR (CI)	*P*†
Controls	161	124 (77.0)	37 (23.0)	1.0	0.0016
Cases	181	110 (60.8)	71 (39.2)	2.16 (1.35–3.47)	
Males					
Controls	49	35 (71.4)	14 (28.6)	1.0	1.0
Cases	54	38 (70.4)	16 (29.6)	1.05 (0.45–2.47)	
Females					
Controls	112	89 (79.5)	23 (20.5)	1.0	0.00027
Cases	123	70 (56.9)	53 (43.1)	2.93 (1.64–5.24)	
Females > 45 years					
Controls	49	39 (77.6)	10 (20.4)	1.0	0.044
Cases	68	42 (61.8)	26 (38.2)	2.41 (1.03–5.65)	
Females ≤ 45 years					
Controls	63	50 (79.4)	13 (20.6)	1.0	0.0017
Cases	55	28 (50.9)	27 (49.1)	3.71 (1.65–8.31)	

OR, odd ratio; CI, confidence interval (95%).
†Two-sided Fisher's exact test.

females over 45 years (OR = 3.71; 95% CI: 1.65–8.31; $P = 0.0017$).

Besides univariate evaluations, we calculated a logistic regression model with gender, age, and *TNFA* (*AA + AG vs GG*), respectively, as possible common explanatory factors for being a 'case'. The estimates for the OR in this model are given in Table 3. The logistic regression confirms that the distribution of age (point estimate = 0.989, 95% Wald CI: 0.975–1.003) and gender (point estimate = 0.998, 95% Wald CI: 0.621–1.604) in cases and controls was similar. In contrast, a significant effect was found for *TNFA-308 AA + AG vs GG* genotype (point estimate = 2.152, 95% Wald CI: 1.332–3.477).

Discussion

Using two different statistical approaches, we found an association between the *TNFA-308 G/A* promoter polymorphism and sensitization to the small chemical allergen PPD. We evaluated that a genotype containing the *TNFA* A allele was significantly more common in individuals with sensitization to PPD than in healthy control subjects, which was especially true for females aged 45 years and older.

Table 3. Odds ratios (*TNFA-308*) for cases and controls calculated by the logistic regression model (maximum likelihood estimates)

Parameter	DF	Estimate	SE	Wald chi-square	$P > \chi^2$
Intercept	1	0.7426	0.3485	4.5394	0.0331
Sex: male	1	−0.00093	0.1210	0.0001	0.9938
Age	1	−0.0111	0.00710	2.4664	0.1163
TNFA AA + AG	1	−0.0111	0.1224	9.8050	0.0017

Odds ratio estimates effect	Point estimate	95% Wald CI
Sex: male *vs* female	0.998	0.621–1.604
Age	0.989	0.975–1.003
TNFA AA + AG vs GG	2.152	1.332–3.477

Sensitization to small molecular weight compounds, such as PPD, is the underlying cause for ACD. Apart from exposure, little is known about factors increasing the risk to develop a sensitization to contact allergens. Predisposing skin conditions and a few epidemiological characteristics, such as gender, age, and race, may play a role (24, 25). Furthermore, genetic variations in relevant important proteins and mediators may influence the individual susceptibility. Previously, an associations between genetic SNPs affecting the individual acetylation status and contact sensitization to PPD have been described (26, 27).

Together with various cytokines and chemokines, TNF-α in particular induces maturation of DCs, such as Langerhans' cells, to immune stimulatory cells and their coordinated migration from the skin to draining lymph nodes (8, 11, 12). Thus, the availability of TNF-α has a major impact on the induction of sensitization and activation of ACD by chemical allergens.

Previous studies indicate that the adenine nucleotide at position -308 in the promoter region of the *TNFA* gene is associated with an increased production of TNF-α (21, 22, 28). The A allele has been found to confer a greater risk for a variety of inflammatory diseases including cerebral malaria (16), mucocutaneous leishmaniasis (17), and asthma (18). Based on these data, we hypothesized that individuals who have the genetic capacity to produce higher levels of TNF-α after encounter with a chemical, such as PPD, may have an increased susceptibility for sensitization and ACD.

Previously, Westphal et al. analyzed the impact of various SNPs in genes altering the production of cytokines including 308 *G/A* and 238 *G/A* in the *TNFA* gene on ACD (20). In accordance with our data, carriers of the *TNFA GA* and *AA* genotype combined were significantly more common in their group of polysensitized persons ($n = 86$), which included individuals sensitized to PPD, compared with healthy controls. However, in the control group genotype, frequencies at the *TNFA-308* locus deviated from Hardy–Weinberg equilibrium and no significant difference for each genotype separately was detected. This might be due to a less representative selection of controls and a limited number of cases defined as polysensitized individuals with positive patch test reactions to both a *para*-compound and at least one other chemically unrelated component. Using both univariate analysis and a logistic regression model, we are now able to complement and extend this data by presenting a genotype distribution for *TNFA-308* in the control group within the Hardy–Weinberg equilibrium and a significant association between individuals sensitized specifically to PPD and carriers of the A allele

($P = 0.0009$). Moreover, we found significantly higher numbers of individuals with *GA* and *AA* genotypes combined ($P = 0.0016$) and *GA* genotypes alone ($P = 0.0037$) among PPD-sensitized patients than individuals with a homozygous *GG* genotype. In accordance with previously published data, individuals with homozygous *AA* genotypes were too rare in both groups for assessing statistical differences.

Our analysis of subgroups revealed in addition that females over 45 years, with *GA* or *AA*-genotypes, have the highest risk for sensitization to PPD, indicating that gender and age might have an impact on the individual susceptibility, which is supported by an overall higher prevalence of ACD in women (25). The age of 45 years as cut-off was chosen because it represents the median age of study participants. Effects of age or gender on ACD are most likely because of age- and gender-specific exposure to certain contact allergens, but may also mirror hormonal influences or age-related changes of the immune status. Interestingly, the *TNFA-308* gene polymorphism and other SNPs in cytokine genes have been associated with aging and age-related diseases (29, 30). Nevertheless, studies including more cases are needed to address this association more properly.

It should be addressed that the validity of this study is influenced by the composition of the study groups, as individuals with a high susceptibility to sensitization, but no exposure to PPD, may be found within the control group. Nevertheless, our findings suggest an association between an increased production of TNF-α because of the *TNFA-308* polymorphism and susceptibility to sensitization and ACD because of PPD.

Notably, Allen et al. found an association between the A allele and a greater risk to develop irritant contact dermatitis to sodium dodecyl sulfate and benzalkonium chloride in Caucasians (19), whereas Dai et al. identified the G allele as a risk factor for trichloroethylene-induced severe generalized dermatitis in Chinese population (31), suggesting that the *TNFA-308 G/A* polymorphism does not only play a role in ACD to PPD, but also in irritant or allergic skin reactions to other chemical compounds. Although TNF-α is clearly involved in these cutaneous immune reactions, it also participates in many other inflammatory processes. Thus, we cannot directly conclude that the higher frequency of *TNFA-308 GA* or *AA* genotype among PPD allergic individuals is unique to PPD sensitization, sensitization to chemical allergens or ACD in general. At most, specificity may result from future studies searching for additional markers based on the hypothesis that the *TNFA-308* polymorphism may be linked to other candidate SNPs within or outside the *TNFA* gene or unknown susceptibility markers. In this respect, it should be mentioned that the *TNFA* gene is located centromerically to HLA-B within the major histocompatibility complex (MHC) class III region. However, up to now, the impact of this association is not clear (32). Another study found an association between genetic variations in the HLA-B region and hypersensitivity reactions to abacavir, a drug used for treatment of HIV-1 infection (33). Additional findings support a hypothesis that a dominant gene marked by HSP70-1 and HSP70-2 within the MHC region, but not necessarily HLA, is associated with disease in different ethnic groups (34). Such associations have been found recently for this SNP and total serum IgE and asthma (35).

Our finding that the *TNFA-308* A allele is associated with an increased risk for the development of sensitization and ACD because of the small chemical PPD is in accordance with data indicating that TNF-α is a key regulator of the initiation of DTH reactions (9). Thus, our study extends these results and demonstrates that the *TNFA-308* genetic polymorphism is most likely a susceptibility factor for contact allergy caused by chemicals such as PPD.

Conflict of interest

None declared.

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